PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Richard J. Cristiano
Dao Nguyen

Serial No.: 09/175,056

Filed: October 19, 1998

For: ENHANCED EXPRESSION OF TRANSGENES

Group Art Unit: 1632

Examiner: D. Nguyen

Atty. Dkt. No.: INRP:021/HYL

CERTIFICATE OF MAILING
37 C.F.R 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231, on the date below:

6/6/00
Date

Richard A. Nakashima

DECLARATION OF RICHARD J. CRISTIANO UNDER 37 CFR § 1.132

Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

I, Richard J. Cristiano, declare that:

1. I am a citizen of the United States, residing in Pearland, Texas.

2. I am the Richard J. Cristiano listed as an inventor along with Dao Nguyen on the above-captioned application.

3. I am also a co-author of Nguyen *et al.*, entitled "Enhancement of gene transduction in human carcinoma cells by DNA-damaging agents," published in *Proc. Amer. Assoc. Cancer Res.* at Volume 37, page 347, March 1996 (attached).

4. F. Spitz, M. Kataoka, S. Wichle and S. Roth are named as co-authors on the above-mentioned Nguyen *et al.* paper but are not named as inventors of the instant application. Dr. Spitz and Dr. Kataoka were clinical fellows, working under my supervision and control, who were involved in the manipulation of animals used in that study. ~~Dr.~~ Ms. Wichle was a Senior Research ~~Associate~~ Assistant, working under my supervision and control, who was involved in animal manipulation and vector preparation. Dr. Roth was the head of the Department of Thoracic and Cardiovascular Surgery at M.D. Anderson, in which the reported study took place. None of these individuals contributed to the conception of the invention claimed in the instant patent application.

5. I hereby declare that all statements made in herein of my own knowledge are true and all statements made on information and belief are believed to be true, and these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 U.S.C. §1001 and may jeopardize the validity of this application or any patent issuing thereon.

5/26/00
Date

Richard J. Christiano

EXPERIMENTAL THERAPEUTICS

#2364 Wednesday, April 24, 1996, 8:00–12:00, Poster Section 14

Development of an adenoviral vector system which confers gene expression which is specific for neoplastic cells. Chung, L., Crystal, R.G., Deisseroth, A.B. *The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, Cornell University Medical Center, New York, NY 10021, and Yale University School of Medicine, New Haven, CT 06520.*

The high transduction efficiency of adenoviral (Ad) vectors has made it one of the most commonly used vectors, but the transduction of this vector into normal cells could limit the use of the Ad vector. The objective of this study was to develop an Ad vector system which would confer upon the tumor cells specific expression of exogenous therapeutic genes. We have generated a recombinant Ad vector, Ad.LP.LacZ and tested it in various cell lines for its targeted expression in neoplastic cells. Ad.LP.LacZ is a replication-deficient Ad vector containing the human L-plastin promoter and the *E. coli* lacZ gene. L-plastin is constitutively expressed in many types of malignant human cells of solid tissues and not expressed in normal tissues, except for the hemopoietic system. As is well known, Ad vectors infect only poorly or not at all early hematopoietic multilineage precursor cells. Infection of Ad.LP.LacZ or Ad.CMV.LacZ (control vector containing the CMV promoter) into the human ovarian carcinoma cell lines, or the human mammary carcinoma cell lines, the normal fibroblast cell lines, and the leukemia cell line, showed tumor-specific expression of β-galactosidase by Ad.LP.LacZ and comparable strength of both (L-plastin and CMV) promoters in tumor cells. In hemopoietic cells such as U937, no measurable β-galactosidase activity was detected from cells infected either by Ad.LP.LacZ or Ad.CMV.LacZ. These results suggest that transcription of therapeutic genes from the Ad.LP-vector system would be restricted to L-plastin expression positive carcinoma cells.

#2365 Wednesday, April 24, 1996, 08:40–08:55, Room 32

Gene therapy targeted by ionizing radiation inhibits tumor growth by decreasing tumor cell mitotic rate and increasing necrosis and leukocyte infiltration. H.J. Mauceri, L.P. Seung, N.N. Hanna, J.D. Wayne, S. Seetharam, D.E. Hallahan, S. Hellman, and R.R. Weichselbaum, *Departments of Radiation and Cellular Oncology and Surgery, Univ. of Chicago, Chicago, IL 60637.*

Intratumoral injection of an adenoviral vector containing radiation-inducible DNA sequences of the Egr-1 promoter linked to a cDNA encoding TNF-α (Ad.Egr-TNF) sensitizes a human radioresistant tumor xenograft (SQ-20B) to the cytotoxic effects of ionizing radiation. Histopathological analysis (day 7) of tumor sections receiving combined treatment revealed a significant increase in both necrosis and leukocyte infiltration and a decrease in tumor cell mitosis. Significant growth delay was observed following 5 injections (2×10^8 PFU Ad.Egr-TNF) and 50 Gy when compared with 2 injections and 50 Gy at both day 14 ($p = 0.05$) and day 31 ($p = 0.01$). Tumors receiving 2 injections + 50 Gy demonstrated significant growth inhibition compared with buffer injected controls ($p = 0.01$), 50 Gy alone ($p = 0.004$) and vector alone ($p = 0.02$, day 17). Increasing the number of injections to 5 (+50 Gy) produced further growth inhibition. Tumors in the combined group (Ad.Egr-TNF + 50 Gy) were significantly smaller compared with buffer injected controls ($p = 0.0001$), 50 Gy alone ($p = 0.015$) and vector alone ($p = 0.0003$, day 17). At 14 days, intratumoral levels of TNF protein were significantly increased following exposure to radiation. These studies suggest TNF and radiation interact to produce growth inhibition through mechanisms involving an increase in both tumor necrosis and leukocyte infiltration and a decrease in tumor cell mitotic rate.

#2366 Wednesday, April 24, 1996, 8:00–12:00, Poster Section 14

Adenoviral mediated p53 gene therapy enhances radiation sensitivity of colorectal cancer cell lines. Spitz, F.R, Nguyen, D., Skibber, J., Meyn, R., Cristiano, R.J., Roth, J.A. *University of Texas M.D. Anderson Houston, Texas 77030*

The p53 tumor suppressor gene has been demonstrated to have a role in cellular response to radiation. Mutations in the p53 gene occur in up to 80% of colorectal cancers. These tumors are often treated with multimodality therapy including radiation. p53 gene transfer into colorectal carcinoma cell lines with p53 mutations (SW620, SW837, KM12L4) was performed utilizing the replication-deficient adenovirus Ad5CMVp53. To evaluate the effect of wildtype p53 expression on radiation sensitivity we performed clonogenic survival assays and tumor growth experiments following Ad5CMVp53 infection. The results indicated that infection with Ad5CMVp53 sensitized the cell lines: the survival for the SW620 line at 2 Gy was reduced from 55% to 23%. FACS TdT analysis indicated increased apoptosis in cells treated with Ad5CMVp53 prior to radiation. Similar results were seen in the SW837 and KM12L4 cell lines. Subcutaneous SW620 xenografts in nude mice were treated in vivo by direct intratumoral injection of AdCMVp53 followed by 5 Gy irradiation. The delay in regrowth to control tumor size of 750 mm^3 was 1 day for 5 Gy, 10 days for Ad5CMVp53; and 24 days for Ad5CMVp53 + 5 Gy indicating synergistic interactions. These data indicate that the delivery of wildtype p53 to cells with p53 mutations increases their radiation sensitivity and this may be accomplished by adenoviral mediated gene therapy.

#2367 Wednesday, April 24, 1996, 09:25–09:40, Room 32

Phase I clinical experience of interleukin-2 (IL-2) gene therapy. RE Sobol, DL Shawler, C Carson, MA Garrett, C Van Beveren, D Mercola LR Smith*, RM Bartholomew*, S Brostoff*, O Dorigo, H Fakhrai, D Carlo*, and I Royston. *Sidney Kimmel Cancer Center and *Immune Response Corporation, San Diego CA 92121.*

We are evaluating IL-2 gene therapy comprising subcutaneous (SC) immunization with a mixture of autologous irradiated tumor cells and IL-2 transduced fibroblasts in patients with colorectal carcinoma or glioblastoma multiforme (GBM). The patients received at least 3 subcutaneous immunizations at 2–4 week intervals. There have been no significant changes in complete blood counts, serum chemistries or urinalyses compared to pre-treatment values. Delayed type hypersensitivity reactions at the sites of the second or subsequent vaccinations were observed in 3/5 patients implying the induction of immunological memory responses. Biopsies of the vaccination sites after the third immunization revealed subcutaneous and dermal perivascular lymphocytic and eosinophilic infiltrates. An anti-tumor immune response mediated in part by CD8+ cytotoxic T cells was demonstrated in the 1 patient analyzed to date. Clinically, 2 patients have had stabilization of previously rising CEA levels during the course of therapy. The patient with the most dramatic DTH like skin reaction has had stabilization of previously enlarging abdominal metastases on CT scan. Tumor necrosis was observed by CT scan in a patient with GBM. In an additional colon cancer patient treated by direct tumor injection of IL-2 transduced fibroblasts, tumor necrosis was also documented by CT scan. These findings suggest that these forms of IL-2 gene therapy are well tolerated and warrant further clinical evaluation.

#2368 Wednesday, April 24, 1996, 1:00–5:00, Room 20

Eradication of established metastatic murine tumors following particle-mediated delivery of IL-12 gene into the skin. Rakhmilevich, A., Turner, J., Ford, M., Sun, W.*, Sondel, P.**, Grota, K., Yang, N-S. *Agracetus Inc., Middleton, WI 53562, *Lurie Can. Ctr. at Northwestern Univ., Chicago, IL and **Univ. Wisconsin Compr. Can. Ctr., Madison, WI.*

We evaluated the antitumor effects resulting from *in vivo* particle-mediated delivery of an IL-12 cDNA expression vector into the skin tissue surrounding and overlying the established intradermal tumors. Direct skin transfection with the IL-12 gene, resulting in the production of sub-nanogram quantities of IL-12 protein in the vicinity of the tumor, induced complete regression of the treated tumors in several murine tumor models. Only 1–4 treatments with IL-12 cDNA-coated particles were required to achieve the regression of established (0.4–0.8 cm in diameter) solid tumors. Moreover, the local IL-12 gene delivery resulted in systemic antitumor effects, leading in some cases to the cure of established visceral metastases. The antitumor effects of IL-12 gene therapy were CD8+ T cell-dependent, and led to the generation of tumor-specific immunological memory. These results suggest that particle-mediated *in vivo* delivery of IL-12 cDNA may offer a simple, non-toxic and useful approach for human cancer gene therapy.

#2369 Wednesday, April 24, 1996, 8:00–12:00, Poster Section 14

Enhancement of gene transduction in human carcinoma cells by DNA-damaging agents. Nguyen, D., Spitz, F., Kataoka, M., Wiehle, S., Roth, JA., Cristiano, R. *Section of Thoracic Molecular Oncology, Department of Thoracic and Cardiovascular Surgery, University of Texas MD Anderson Cancer Center, Houston, Texas.*

Recombinant viruses are popular vectors for gene therapy of benign or malignant disease. Strategies aimed at maximizing target cell transduction of therapeutic genes without increasing the viral titers may minimize vector-related toxicity. Incubation of H1299 human lung cancer cells with cis-diamminedichlorocisplatin (CDDP) prior to infection with an adenovirus expressing βGal (Adv/βGal) led to an enhancement of reporter gene transduction that was 2 to 2.5 fold greater than non-treated cells. Maximal expression of the βGal gene occurred only in cells that were treated with 0.016 to 0.062 μg/ml of CDDP 2 days prior to gene transfer. Increased gene transduction was observed in other cancer cells but not in primary normal human bronchial epithelial cells. It was also noted in CDDP-treated H1299 cells transfected by other gene delivery systems (lipofectamine or conjugated Adv/DNA complex) carrying the βGal plasmid. Similar exposure of H1299 cells to DNA-damaging agents but not to other classes of antineoplastic drugs resulted in the same degree of elevated reporter gene transduction. In vivo βGal gene transduction was increased in H1299 tumors injected with Adv/βGal on days 2 and 4 but not day 6 following intraperitoneal CDDP administration. In conclusion, exposure of malignant cells but not normal cells to CDDP resulted in a dose-related, time course-dependent, vector-independent enhancement of foreign gene transduction efficiency. These results suggest a new, more effective strategy of gene therapy for malignant disease using sequential combination of CDDP and adenovirus-mediated gene transfer.

#2370 Wednesday, April 24, 1996, 09:10–09:25, Room 32

Gene therapy for lung cancer: Enhancement of tumor suppression by a combination of systemic cisplatin and adenovirus-mediated p53 gene transfer. Nguyen, D., Wiehle, S., Koch, P., Roth, JA., Cristiano, R. *Section of Thoracic Molecular Oncology, Department of Thoracic and Cardiovascular Surgery, University of Texas M.D. Anderson Cancer Center, Houston, Texas.*